List of publications from the research team on the study of the use of extracorporeal affinity adsorption in immunolocalisation and immunotherapy of tumours.

Scientific papers

Doctoral dissertations

Scientific papers:

1. Nilsson R, Lindgren L, and Lilliehorn P
Extracorporeal immunoadsorption therapy on rats. In-vivo depletion of specific antibodies.
Clin.Exp.Immunol. 82:440-444 (1990)

2. Norrgren K, Strand SE, Nilsson R, Lindgren L, and Lilliehorn P.
Evaluation of extracorporeal immunoadsorption for reduction of the blood background in diagnostic and therapeutic applications of radiolabelled monoclonal antibodies.
Antibody Immunoconj. Radiopharm. 4:907-914 (1991)

3. Norrgren K, Strand S.E, Ingvar C.
Contrast enhancement in RII and modification of the therapeutic ratio in RIT: A theoretical evaluation of simulated extracorporeal immunoadsorption.
Antibody Immunoconj. Radiopharm. 5:61-73 (1992)

4. Norrgren K, Strand SE, Nilsson R, Lindgren L, and Sjögren H.O.
A general extracorporeal immunoadsorption method to increase the tumor-to-normal tissue ratio in radioimmunoimaging and radioimmunotherapy.
J.Nucl.Med. 34: 448-454 (1993) RM 845 J78

5. Garkavij M, Tengvall J, Strand SE, Norrgren K, Nilsson R, Lindgren L, Sjögren HO.
Improving radioimmunotargeting of tumors: Variation in the amount of MAb L6 administered, combined with an immunoadsorption system (ECIA).
Acta Oncol. 32:853-859 (1993)

6. Strand SE, Norrgren K, Garkavij M, Lindgren L, Nilsson R, Sjögren HO, Tengvall J.
Development of a general extracorporeal immunoadsorption method to increase tumor-to-nontumor ratio.
Cancer (3 suppl) 73:1033-1037 (1994)

7. Norrgren K, Garkavij M, Lindgren L, Nilsson R, Strand S.E, Tennvall J, Sjögren H.O
Extracorporeal immunoadsorption of ^{125}I-labeled monoclonal antibody L6.
Antibody Immunoconj. Radiopharm. 7:29-42 (1994)

8. Garkavij M, Tennvall J, Strand SE, Norrgren K, Nilsson R, Sjögren HO.
Improving radioimmunotargeting of tumors: The impact of a preloading of unlabeled L6 MAb on the biodistribution of ^{125}I-L6 in rats.
J.Nucl.Biol.Med. 38: 594-600 (1994)

9. Strand S.E, Ljungberg M, Tennvall J, Norrgren K, and Garkavij M.
Radioimmunotheraphy dosimetry with special emphasis on SPECT quantification and extracorporeal immunoadsorption.
Medical and Biological Engineering&Computing 32:551-561 (1994)

10. Garkavij M, Tennvall J, Strand SE, Norrgren K, Lindgren L, Nilsson R, Sjögren HO
Enhanced radioimmunotargeting of ^{125}I-L6-biotin monoclonal antibody (MAb) by combining preload of cold L6 Mab and subsequent immunoadsorption in rats.
Cancer Research 55 (23 Suppl.):5874-5880 (1995)

11. Garkavij M, Tennvall J, Strand SE, Nilsson R, Lindgren L, Chen J, Isaksson M, Eriksson H, Sjögren HO.
Extracorporeal immunoadsorption from whole blood based on the Avidin-Biotin concept: Evaluation of a new method.
Acta Oncol., 35(3):309-312 (1996) ADONIS

12. Chen J, Strand S.E, Sjögren H.O.
Optimization of radioiodination and biotinylation of monoclonal antibody chimeric BR96: An indirect labeling using N-succinimidyl-3-(tri-n-butylstannyl)benzoate conjugate.
Cancer Biotherapy & Radiopharmaceuticals. 11:217-226 (1996)

13. Garkavij, Tennvall J, Strand S.E, Sjögren H.O, Chen J, Nilsson R, Isaksson M.
Extracorporeal whole-blood immunoadsorption enhances radioimmunotargeting of iodine-125-labeled BR96-biotin monoclonal antibody.
J.Nucl.Med. 38(6):895-901 (1997)

14. Tennvall J, Garkavij M, Chen J, Sjogren HO, Strand SE.
Improving tumor-to-normal-tissue ratios of antibodies by extracorporeal immunoadsorption based on the avidin-biotin concept: development of a new treatment strategy applied to monoclonal antibodies murine L6 and chimeric BR96.
Cancer 80(12 Suppl.):2411-2418 (1997)

15. Chen JQ, Strand SE, Tennvall J, Lindgren L, Hindorf C, Sjogren HO
Extracorporeal immunoadsorption compared to avidin chase: enhancement of tumor-to-normal tissue ratio for biotinylated rhenium-188-chimeric BR96.
J.Nucl.Med. 38(12):1934-1939 (1997) Rm 845 J.78

16. Chen JQ, Strand SE, Tennvall J, Hindorf C, Sjogren HO
Biodistribution ans pharmacokinetics of biotinylated 188Re-chiBR96 in colon carcinoma isografted rats.
Tumor Targeting 3:87-95 (1998)

17. Garkavij M, Tennvall J, Ohlsson T, Lindgren L, Hindorf C, Sjogren HO, Strand SE
Comparison of 125I-and (111)In-labelled monoclonal antibody BR96 for tumor targeting in combination with extracorporeal immunoadsorption.
Clin.Cancer Res. 5(10 suppl.):3059-3064 (1999)

Doctoral dissertations:

Christian Ingvar (1990)
Radiolabelled monoclonal antibodies. An experimental study in the nude rat heterotransplanted with malignant melanoma.
Department of Surgery, University of Lund.

Kristina Norrgren (1993)
Radiolabeled monoclonal antibodies. Development of a new method to remove circulating activity - Diagnostic applications and implications for therapy.
Department of Radiation Physics, University of Lund.

Michael Garkavij (1996)
Improving radioimmunotargeting of tumors. The impact of extracorporeal immunoadsorption and preload in rats.
Department of Oncology, University of Lund.

JianQing Chen (1997)
Radiolabeling and biotinylation of internalizing monoclonal antibody chimeric BR96. Potential use for

extracorporeal immunoadsorption with enhanced tumor radioactivity retention of Iodine, Indium and Rhenium. Department of Radiation Physics, University of Lund

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9

Extracorporeal Techniques in Radioimmunotherapy

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I. INTRODUCTION

A major problem in achieving effective immunotargeting is the poor tumor-to-blood activity ratio which rarely reaches values sufficiently high to give effective radioimmunotherapy (RIT) or permit accurate diagnostic radioimmunoimaging (RII). This is of particular importance in RIT, where the toxic effect of conjugates on critical organs (marrow) is normally a limiting factor due to high absorbed doses from the relatively long circulation time of intact monoclonal antibodies (MAbs). Although MAb fragments such as Fab or F(ab')2 are cleared more rapidly from the blood and penetrate more easily into solid tumors than the intact antibodies, most MAb fragments do not accumulate sufficiently in tumors to achieve the desired therapeutic effect (I).

There are currently a number of methods described which aim to increase the tumor-to-blood activity ratio by artificially decreasing the amount of the radioimmunoconjugate (RIC) in the blood while retaining the level of the conjugate in the tumor. The various strategies to enhance the tumor-to-normal tissue absorbed dose ratios (including extracorporeal technique) have been reviewed (I). This chapter will focus on extracorporeal techniques.

In view of this, it is not surprising that the use of various extracorporeal techniques for the removal of circulating RIC has been contemplated (2-4). Our group has proposed the extracorporeal immunoadsorption (ECIA) technique (5). Extracorporeal techniques are generally employed for the removal of pathogenic or otherwise undesirable agents from the bloodstream of patients with various severe diseases. In numerous cases it has been proven efficient and sometimes life saving. Most notable is the success of dialysis in the treatment of renal failure (>700,000 patients on chronic treatment worldwide).

II. PRINCIPAL WAYS FOR EXTRACORPOREAL DEPLETION

There are two principal ways to control levels of systemically administered immunoconjugate by extracorporeal means; either the patient's plasma is continuously or stepwise removed and replaced by donated plasma or by albumin solutions, or the patient's plasma or whole blood is cleared by the use of specific adsorption devices. Both these methods have been explored clinically. Any technique where blood is exchanged by continuous or discontinuous withdrawal follows an exponential curve with respect to clearance efficiency (Fig. 1). Removing exogenous or endogenous toxic agents from the circulation by plasma exchange in an online setting is standard procedure for a number of diseases and syndromes. However, such treatments are not without serious drawbacks. If a patient's plasma is replaced by albumin solution, rarely more than one plasma volume can be processed due to the fact that essential components in plasma, such as coagulation factors, must not be decreased by more than that. Hence, it is inconceivable that more than 60% to 65% of nonbound

immunoconjugate could be removed from the patient's plasma through such means. If on the other hand the patient's plasma is exchanged for donated human plasma, hypersensitivity may occur. There is also a risk of contamination.

A more attractive method is the selective removal of undesirable agents from blood circulation, with a simultaneous return of the purified blood to the patient, thus avoiding loss of essential blood components and eliminating the need for replacement solutions. These methods are typically based on the principle of affinity adsorption, although size and physical properties could also be utilized. In extracorporeal affinity treatment, a substance selective for the component to be removed is covalently bound to a matrix through which the blood or plasma is passed (Fig. 2).

Clinical extracorporeal adsorption systems for processing human plasma are available for the removal of immunoglobulin by the use of Protein-A in the treatment of autoimmune diseases (6) or to avoid early rejection of transplanted organs (7), and for the removal of low-density lipoprotein (LDL), in the treatment of hypercholesterolemia by the use of immobilized anti-LDL antibodies (8).

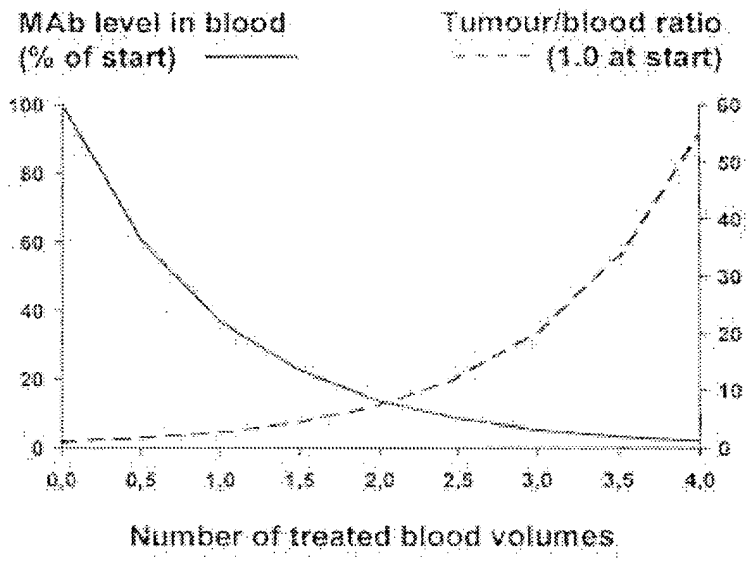


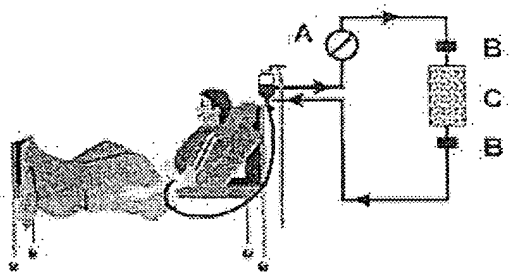
Figure 1 A theoretically calculated curve illustrating the improvement of the tumor-to-blood ratio as a function of number of blood volumes processed. The concentraion of immunoconjugate at start of the affinity adsorption is set to 100%, and the tumor-to-blood ratio is assumed to be 1.0. After three blood volumes have been processed, abort 95% has been removed and the ratio has increased to 20.

Any device used with the purpose to specifically remove RIC must rely on the presence of structural entities on the immunoconjugate accessible for binding to the device. These structural entities can either be an integral part of the immunoconjugate or be artificially conjugated to the immunoconjugate prior to administration to the patient. An example of the former is the use of immobilized antibodies directed against specific and accessible epitopes present on the immu- noconjugate. An extracorporeal system utilizing immobilized anti-isotypic (antispecies) antibodies directed against immunoconjugate based on mouse MAbs has been developed and clinically evaluated (9).

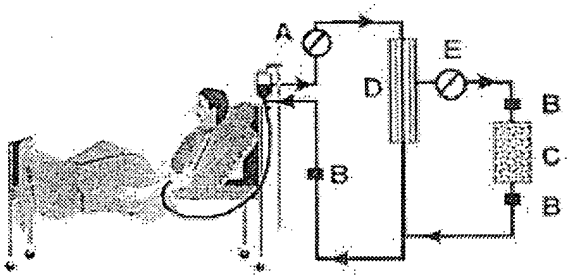
Over the past 5 or 6 years most pharmaceutical companies in the field have focused their efforts on alternatives to mouse monoclonal antibodies for various types of immunotargeting. It is generally acknowledged that xenotypic monoclonal antibodies give rise to production of human antibody directed against the administered monoclonal antibodies (10). To minimize these effects attempts have been made to humanize part of the mouse monoclonal antibodies by replacing mouse structures for the corresponding human structures (II). In the long term there is little doubt that new generations of immunoconjugates will be based on monoclonal antibodies with structures closely related to that of human IgG. Consequently, such exogenous humanized antibodies (foreign) might differ from the endogenous human antibodies (own) only with respect to the specific antigenbinding region.

Any general affinity adsorption device has to be based on artificially introduced characteristics of the exogenous antibodies in order to differentiate between own and foreign IgG. This can be achieved by the labeling of the exogenous antibody with affinity ligands (12,13). Such affinity ligands should exhibit a high affinity as well as high specificity toward a given receptor immobilized to the adsorbent. Another prerequisite is that the affinity labeling of the immunoconjugate must not significantly alter the tumor-binding properties of the monoclonal antibodies, or affect the biodistribution or enhance the immunogenicity of the conjugate. The stability of the conjugate is also important since successful extracorporeal removal of non-target-bound conjugates will be dependent on the presence of accessible affinity ligand moieties on the conjugate. Furthermore, if the affinity ligand is an endogenous substance, the content in human blood must be low so as not to interfere with the adsorption of the conjugate to the device. Furthermore, the immobilized receptor utilized must not interfere with

the blood through activation or inhibition of vital physiological processes. The use of biotin (vitamin H) as affinity ligand in conjunction with immobilized avidin/streptavidin fulfill most of the above described criteria.



Adsorption system for whole blood



Adsorption system for plasma

Figure 2 Principal outline of the affinity-adsorption systems for the treatment of whole blood and plasma, respectively. (A) Blood pump. (B) Air detector with pressure monitor. (C) Adsorption device. (D) Plasma separation device (plasma filter or online centrifuge). (E) Plasma pump.

III. RADIOLABELING AND BIOTINYLATION OF IMMUNOCONJUGATES

A successful combination of procedures for biotinylation and radiolabeling of immunoconjugates is a prerequisite for the use of ECA T based on the biotin-avidin concept.

Many papers regarding the optimal radionuclide for radioimmunotherapy have been published (14-17). Due to its familiarity, relatively simple labeling, appropriate physical half-life, ready availability, and low cost, ^{131}I has been used in many experiments and clinical RIT studies. In our experience, the appropriate means of combining radiolabeling and biotinylation varies for different types of MAbs. Direct iodination has been successfully used for MAb L6 radiolabeling in conjunction of biotinylation (12,18) and for other MAbs by the Paganelli group (19), whereas it is less straightforward to apply these techniques for BR96 (20). Instead, the use of N-succinimidyl-3-(tri-n-butylstannyl)benzoate (NSTBB) for BR96 iodination, followed by subsequent biotinylation with N-hydroxysuccini- mido-biotin, has proven more successful (20,21). Chelates such as SCN-Bz-CHX- A-DTPA and TFP MAG2-GABA (tetrafluorophenyl mercaptoacetylglycylglycylgamma-aminobutyrate) have been found appropriate for indium and rhenium labeling of biotinylated BR96 (22,23). The maintenance of tumor cell binding properties was confirmed on tumor cell lines in vitro. The in vivo stability of the radiolabeled and biotinylated BR96 was examined by determining free radioactivity and the binding to avidin-agarose in plasma. The biodistribution of the radiolabeled BR96 with or without biotin was further compared in the colon carcinoma isografted Brown Norwegian (BN) rat model. In Table 1, a comparison of biodistribution of the biotin-coupled and non-biotin-coupled radiolabeled BR96 (with 1251, 188Re, or 1111n) at 48 h postinjection in the colon carcinoma isografted BN rats is given. As seen in the table, there is a close resemblance in activity uptake in tumors and normal tissues for nonbiotinylated and biotinylated MAb. Biotinylation of proteins such as immunoglobulins can be achieved through various means. The amino groups in proteins can be

conjugated by the use of biotinyl-p-nitrophenyl esters or biotinyl-N-succinimide esters. The coupling can also be achieved by the use of carbodiimide and equivalent coupling reagents. In all these cases the biotinyl group will be linked to the ?-amino groups of lysine residues forming a biocytin derivative, although a limited number of a-amino groups may also be conjugated. The combined radioiodination and biotinylation of immunoconjugates have been extensively investigated (19). However, exploiting primary amino groups for biotinylation purposes may cause some problem in the cases where the same amino groups are needed for the radionuclide labeling.

Table 1 Biodistribution of the Biotin-Coupled and Non-Biotin-Coupled Radiolabeled BR96 (with ^{125}I, ^{188}Re, or ^{111}In) at 48 h Postinjection in Colon Carcinoma Isografted BN Rats (% injected activity per gram tissue)

	^{125}I-BR96	^{125}I-BR96-biotin	^{188}Re-BR96	^{188}Re-BR96-biotin	^{111}In-BR96	^{111}In-BR96-biotin
Tumor (im)	2.19 ± 0.40	1.79 ± 0.74	2.07 ± 0.62	1.78 ± 0.37	2.57 ± 0.32	2.68 ± 0.38
Tumor (sr)	2.91 ± 0.17	2.51 ± 0.69	2.76 ± 0.66	2.41 ± 0.76	6.54 ± 1.36	5.61 ± 0.79
Testicle	0.17 ± 0.01	0.15 ± 0.00	0.16 ± 0.02	0.14 ± 0.02	0.41 ± 0.09	0.43 ± 0.05
Liver	0.52 ± 0.03	0.49 ± 0.04	0.27 ± 0.11	0.29 ± 0.09	1.14 ± 0.18	1.13 ± 0.23
Spleen	0.31 ± 0.02	0.28 ± 0.03	0.18 ± 0.04	0.19 ± 0.02	0.32 ± 0.04	0.31 ± 0.02
Kidney	0.28 ± 0.03	0.28 ± 0.05	0.48 ± 0.04	0.49 ± 0.05	0.54 ± 0.06	0.49 ± 0.05
Colon	0.24 ± 0.05	0.22 ± 0.02	0.32 ± 0.08	0.32 ± 0.08	0.42 ± 0.18	0.52 ± 0.08
Lung	0.31 ± 0.02	0.30 ± 0.00	0.37 ± 0.09	0.34 ± 0.04	0.63 ± 0.09	0.60 ± 0.04
Bone marrow	0.24 ± 0.02	0.16 ± 0.11	0.21 ± 0.03	0.20 ± 0.05	0.35 ± 0.16	0.40 ± 0.12
Blood	0.64 ± 0.09	0.58 ± 0.05	0.82 ± 0.13	0.82 ± 0.09	1.12 ± 0.15	1.08 ± 0.09

This may not severely hamper the use of these agents, provided that biotinylation is accomplished prior to the radiolabeling. It is probably of minor importance whether the radionuclide is buried inside the IgG molecule or exposed on the surface. The biotin groups, however, must at least to some extent be available on the surface of the immunoglobulin in order to be captured by the immobilized avidin. Although natural biotin carries a short spacer of four carbon atoms, which together with the four carbon atoms of the lysine residue side chain should distance the biotin double ring from the backbone structure of the immunoglobulin, there might still in some cases be an advantage to introduce spacers of various length.

Alternative ways of preparing biotin derivatives reacting with groups other than amino groups are also commonly used. Among these are biotinyl hydrazide which reacts with sugar residues and biotinyl-bromoacetyl hydrazide or biotin maleimide, which reacts with sulfhydryls and other strong nucleophiles. Biotinyldiazoanilide can be used to conjugate biotin to phenol or imidazole functions. To achieve sufficiently high avidin binding capacity without significant alteration of the antigen-binding properties of the immunoconjugate, the number of conjugated biotin moieties should be limited but high enough to ensure that nearly all individual MAbs carry at least one biotin residue. The optimal number will most likely vary from MAb to MAb and depend on the conditions for extracorporeal removal, but something in the range of three to five biotin residues per MAb will probably be appropriate.

Although there is some controversy regarding the physical states of biotin in human blood, in a recent study of 10 healthy persons of both sexes, the average total amount of biotin was found to be 483 pmol/L (276 to 785 pmol/L) (24). Thus, on average a patient having 6 L blood will carry about 15 µg of extracellular biotin. This amount of extracellular biotin should be capable of blocking about 1 mg of immobilized avidin, assuming that all four binding sites for biotin are available on the immobilized avidin. Hence, the presence of naturally occurring extracellular biotin is unlikely to interfere with the efficiency of the extracorporeal depletion. Due to the short duration of biotin depletion it is very unlikely that the patients will show any manifestations of biotin deficiency.

The stability of the biotinyl linkage is of utmost importance, since this concept of extracorporeal removal will rely on the presence of biotin residues on the MAb. Naturally occurring biotin is recycled from biocytin by the cleavage into lysine and biotin, through the activity of a group of enzymes called biotinidase (25). However, these enzymes are sensitive to steric hindrance near the biotin carboxyl function, making it unlikely that biotin groups directly linked to the protein would be removed from the MAb molecule prior to the extracorporeal depletion (26). The relatively short time interval of the immunoconjugate circulating in blood prior to the extracorporeal removal should also be considered.

IV. DOSIMETRIC CONSIDERATIONS AND THERAPEUTIC RATIO

DeNardo et al. (27) emphasize that the imaging efficacy is dependent on amount of radionuclide in target and normal tissues; i.e.,

thetumor-to-normal-tissue activity ratio at the time point of imaging has to be as large as possible, while the therapy efficacy is dependent on cumulative activity in target and normal tissues; i.e., the tumor-to-normal-tissue residence time- or absorbed dose ratio has to be as large as possible.

The absorbed dose to different organs/tissues in radionuclide therapy is usually calculalated using the MIRD S-formalism (28). Mean absorbed dose in the target region, D is calculated as the product of the cumulated activity in the source region, *A,* and the S-value. Here are the simplified equations describing the MIRD formalism presented (summation for type of radiation and summation of contribution from more than one source region are omitted).

T $\overline{D} = \tilde{A} \cdot S$ (1)

nulated activity, *A,* is determined from the biologic parameters (the source region's uptake and clearence) and is calculated as the time integral of the time-activity curve for an organ or tissue (the source organ):

$$\tilde{A} = \int_0^\infty A(t)dt \qquad (2$$

The physical parameters is gathered in the S-value:

$$S = ? \cdot \frac{\phi}{m} \qquad (3$$

where ? is emitted energy per decay of the radionuclide, ? is absorbed fraction, and *m* is the mass of the target region. (See Chapter 2 in this volume for a more extensive review of the MIRD S-formalism.)

When radionuclide therapy is combined with ECAT the calculation of mean absorbed dose has to be done in a slightly modified way. The S-value for a given region remains the same whether ECAT is performed or not, but the cumulated activity is changed (Fig. 3). Mean absorbed dose in an organ/tissue after extracorporeal imrnunoadsorption, D_{ECAT} is then calculated as:

(4) $\overline{D}_{ECAT} = \tilde{A}_{ECAT} \cdot S$

The cumulative activity when extracorporeal immunoadsorption is performed, A_{ECAT}, is calculated as the time integral of the time-activity curve as before, but the integration should be divided into three different time periods - the first period describing biokinetics from injection point to start of ECAT, the second describing clearance during ECAT, and a third period for the biokinetics from stop of ECAT to infinity (29).

(5) $$\tilde{A}_{ECAT} = \int_0^{ECATstart} A_{before\ ECAT}(t)dt + \int_{ECATstart}^{ECATstop} A_{ECAT}(t)dt + \int_{ECATstop}^{\infty} A_{after\ ECAT}(t)dt$$

The curve describing the removal of activity during ECAT, *AECAT(t),* should be determined experimentally, with a scintillation camera as described in an experimental model (13) or by blood samples measured with a gamma counter as de- scribed for patients (30). If this is not possible during ECAT, a monoexponential decrease of activity could be assumed if the activity is known immediately before and after performance of ECAT (31). This assumption, of course, introduces an uncertainty in the cumulated activity, but since the time to perform ECAT is short compared to the whole integration time, this error would be small and could probably be neglected.

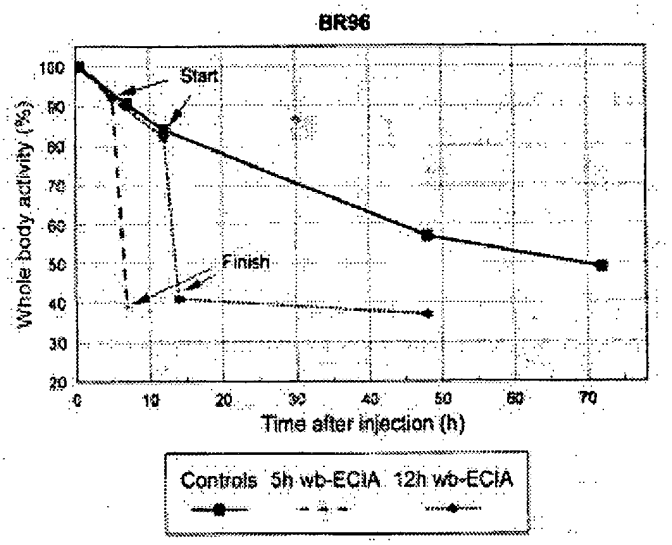


Figure 3 Whole-body activity curves after injection of ^{125}I-chiBR96-biotin in either controls or rats subjected to wb-ECAT.

An often reported quantity is the therapeutic index, i.e., the ratio of absorbed doses to tumor and to critical organs. Therapeutic index is reported to compare the efficency of radioimmunotherapy with and without ECAT. The reason for performing ECAT is to maintain a high absorbed dose to the tumor while decreasing absorbed dose to critical organs, so the therapeutic index should be higher when combining radioimmunotherapy with ECAT than radioimmunotherapy alone. Values of therapeutic index obtained both from theoretical estimations and from experiments reported in the literature are given in Table 2.

Reported values on therapeutic characteristics for radioimmunotherapy with and without ECAT vary considerably, as seen in Table 2. This variation is due to several factors, but the main reasons are different ECAT systems and different theoretical models employed. The interval between injection of radioimmunoconjugate and start of ECAT, the choice of critical organ for evaluating therapeutic index, and the choice of radionuclide for therapy, all will influence the therapeutic index, as is elucidated in the theoretical models.

A compartmental model describing experimental data on the pharmacokinetics of an antibody labeled to ^{111}In and the removal of activity during ECAT was developed by Hartman et al. (34). Their simulation of starting time of ECAT postinjection shows a decrease of percent removed activity with an increased onset time of ECA T postinjection and the simulation of duration of ECAT shows increase in percent removed activity with an increase in duration time of ECAT .

Norrgren et al. (33) have constructed a compartmental model from biokinetic data to theoretically evaluate the efficacy of ECAT .The model involves 13 compartments and for each compartment the influence of ECAT on the time- activity curve for an organ is simulated. A simulation of treatment efficiency for different starting times of ECA T shows that the highest ratio of tumor and blood activity is obtained if the starting time for ECAT corresponds to the time for the highest activity uptake in the tumor. Norrgren et al. (33) pointed out that the therapeutic ratio varies depending on which critical organ is chosen, tumor-to-whole-body ratio is, for example, higher than tumor-to-bone-marrow ratio.

Table 2 Theoretically and Experimentally Obtained Therapeutic Characteristics

Experimental model	Radio-nuclide	Therapeutic characterisitics	Reference
Patients, anti-immunoglobulin system	^{111}In	$\bar{A}_{blood}$ mean decrease is 21% and 43% (two calculation models)	(29)
Theoretical simulation Extravascular tumor	^{123}I ^{125}I ^{131}I	*T/BM = 8; T/BM_{ECAT} = 7 T/BM = 0; T/BM_{ECAT} = 2 T/BM = 23; T/BM_{ECAT} = 44	(32)
Compartment model Theoretical model	^{211}At ^{90}Y ^{125}I	*T/WB_{ECAT} = 0.8; T/BM_{ECAT} = 0.15 T/WB_{ECAT} = 1.75; T/BM_{ECAT} = 1.25 T/WB_{ECAT} = 0.75; T/BM_{ECAT} = 0.4	(33)
Patients, anti-immunoglobulin system	^{111}In	D_{BM} decreased 29–56% while D_T remained the same with ECAT	(30)

* T/WB: Ratio of absorbed dose to tumor and whole body; T/BM: ratio of absorbed dose to tumor and bone marrow.

DeNardo et al. (27) have done a simulation to optimize adsorption onset time regarding ratio of tumor to blood absorbed dose. From their simulation it is confirmed that blood clearance rate, tumor uptake and clearance rate, and radio- nuclide decay influence the optimal starting time of the adsorption procedure.

Some models show an increase in plasma activity after termination of ad- sorption (33,34). This increase is probably a redistribution of activity from the extravascular compartment, due to the rapid decline in plasma activity during ECAT. If the duration time of the adsorption is increased, the simulation shows a larger redistribution of activity after end of ECAT procedure (34).

Sgouros (32) has calculated absorbed dose to the tumor as a function of tumor radii, showing that different radionuclides give different homogeneity of absorbed dose. The simulations by Sgouros of radioimmunotherapy in combi- nation with plasmapheresis show for example a very high therapeutic index when ^{125}I is used in a model with a hematologic tumor; however, the absorbed dose in the tumor is highly nonuniform. As almost no absorbed dose is delivered to the central part of the tumor, the efficacy of the therapy will be low. This understanding indicates the need for microdosimetric considerations rather than using mean absorbed dose. By varying the amount of MAb administered in the simulation, Sgouros shows that a larger amount MAb results in a more uniform absorbed dose profile in the tumor. The latter should be followed by ECAT to decrease the absorbed dose to normal tissues and increase the therapeutic ratio.

V. STUDIES OF EXTRACORPOREAL AFFINITY SYSTEMS ON ANIMALS

Experimental studies with ECAT in animals have been reported by our group. Athymic rats as well as euthymic Brown Norwegian (BN) rats were used in these studies. The athymic rats were inoculated with human melanoma tumor (UMTlO) subcutaneously and intramuscularly, or with human lung carcinoma (H2981) intramuscularly and beneath the left kidney capsule. The BN rats were injected intramuscularly and beneath the left kidney capsule with colon carcinoma chemically induced in the same strain. Details of these experiments are published elsewhere (12,35,36).

Three radioiodinated and biotinylated MAbs, murine 96.5, murine L6 or chimeric (chi) BR96, and ^{188}Re or ^{111}In and biotinylated BR96, were employed (12,35-37). Quality control of radiolabeled and biotinylated MAbs included cell binding capacity, the presence of free isotopes in the preparations as well as binding to avidin gel prior to injection (12).

Before ECAT was performed, the animals underwent arterial (a.carotis communis) and venous (v.jugularis) catheterization. In studies of ^{125}I-96.5-biotin and ^{125}I-L6-biotin, the catheters were connected to the ECAT system 24 or 48 h postinjection, and the blood was then pumped through a hollow fiber plasma filter. The separated plasma was passed through the adsorption column, which contained 1 to 1.5 mL of avidin agarose at a flow rate of 0.2 mL/min, and the processed plasma was then mixed with the blood cells and returned via the venous catheter. During a 3-hour treatment three plasma volumes were passed through the column.

There are some appearent disadvantages in using a p-ECAT system in rats-hemolysis during plasma separation, hypervolemic effects, the complexity of tubing connections, and the long time required for preparation. An alternative avenue using ECA T was therefore explored (Fig. 2). This system enables direct adsorption of biotinylated MAb from unseparated blood, and includes only a peristaltic blood pump, an adsorption column, and connecting tubings with a drop chamber. The adsorption column (1.5 mL) contained avidin covalently linked to larger-size agarose beads. Blood was pumped from the arterial catheter through the adsorption column at a flow rate of 1.0 to 1.5 *mLlmin.* The adsorption treat- ment lasted 2 h.

The rats were divided into a control group (to be given radiolabeled MAb only) and an ECAT group. The p-ECAT was performed 24 h after the injection of ^{125}I-96.5-biotin or ^{125}I-L6-biotin. Wb-ECAT was accomplished 12 h postinjection of ^{125}I-chiBR96-biotin. The difference in timing was dependent on the MAb kinetics and not the selected adsorption technique.

Blood sampling and whole-body imaging were performed immediately after the injection of MAb, just before start of ECAT , immediately after termination of adsorption, and at 48 and 72 h after the injection of MAb. The animals were killed either after termination of the adsorption or 24 h (L6) or 33 h (chiBR96) after completion of the procedure.

At dissection, the tumors as well as several organs (bone marrow, liver, lungs, right kidney, thigh muscles, pancreas, bowel, spleen, stomach, lymph nodes, and thyroid) were removed, weighed, and measured for activity .The activity uptake was expressed in percent of the injected activity per gram tissue (%IAlg), corrected for decay. *TIN* uptake ratios were based on the %IA/g of tumor and %IA/g of normal tissue.

Immediately after p-ECAT, whole-body activity for ^{125}I-L6-biotin was reduced by 38%. Scintigraphic visualization of implanted tumors in the region of the kidney was greatly improved and was in several cases only possible after ECAT. Approximately 80% to 95% of the circulating plasma activity was removed. The plasma activity was found to increase only slightly during the 24 h following the completion of ECAT. The ECA T procedure improved T *IN* ratios by a factor of 2.5 to 8.4 (median 3.15) for lungs, and 6.3 to 35.7 (median 12.6) for bone marrow. The reduction of activity in liver, lung, bone marrow, and kidney 24 h after ECAT was more pronounced than that in tumors, so the T/N ratios were still elevated compared with untreated rats at the same time postinjec tion.

Similar results for BR96 were found for both iodinated and rhenium-labeled biotinylated BR96. By using wb-ECAT, radioactivity of whole body was , by about 50% (Fig. 3) and plasma activity by about 85%. Both directly completion of wb-ECAT and 33 h later, the displayed activity uptake in was not significantly different from that of control animals ($P > .05$), and had approximately similar time-activity curves. The activity in bone marrow, liver, kidney, lung, pancreas, and bowel directly after completion of wb-ECAT was reduced by 40%. The activity removed by ECAT from normal organs correlated very well with blood content in respective organ (12). Activity uptake ratios after completion of ECAT are shown in Figure 4.

The present studies showed that ECAT was applicable to different MAbs , by different isotopes, and resulted in substantially improved tumor-to-normal-tissue ratios.

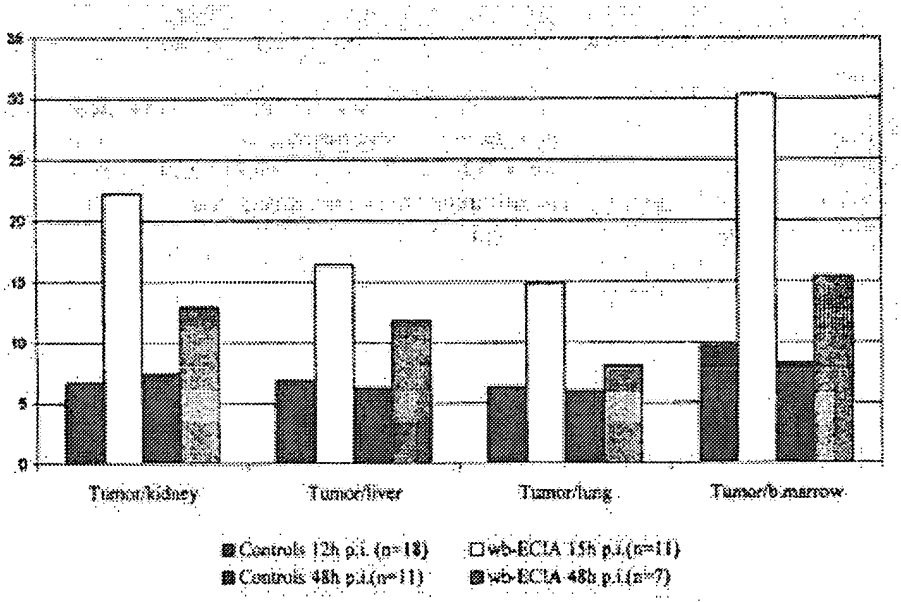


Figure 4 Improvement of tumor-to-normal-organ activity ratios after wb-ECAT in com- parison with corresponding controls.

VI. CLINICAL CONSIDERATIONS OF EXTRACORPOREAL TREATMENTS

Extracorporeal systems utilizing immobilized anti species antibodies has been evaluated for antibody tumor imaging. In 21 patients treated with a device comprising goat antimouse MAbs, there was substantially improved image contrast (9,29,30). The same technique has been exploited in RIT, albeit to a limited number of patients. By using the goat antimouse antibody adsorption device to remove non-tumor-bound ^{131}I-labeled L6 in the treatment of breast cancer and ^{131}I-labeled Lym-1a monoclonal antibody directed to B-celllymphoma, De Nardo et al. reported a depletion of 65% to 85% of the activity in total body, whereas tumor radioactivity was not significantly altered (27,38,39).

Hence, the clinical feasibility of the above described procedure has been demonstrated and the predicted reduction in plasma activity and bone marrow absorbed dose has been verified. However, the specificity of this goat antimouse antibody clearly dictates the need for a more general technique which is not limited to the adsorption of antibodies of mouse origin. Principally such adsorbents could be based on immobilized anti-idiotypic antibodies. However, if the concept is going to gain widespread use it is not feasible to develop an affinity adsorption system specific for only a particular type of antibody. This can be circumvented by the labeling of the immunoconjugate with affinity ligands such as biotin.

After administration, the immunoconjugate will gradually begin to metabolize. Any adsorption technique based on immobilized antibodies directed against specific epitopes of the immunoconjugate will depend on the intact structure of the epitope, whereas in the case of removing affinity-labeled antibodies it is sufficient that the cytotoxic moiety is adjacent to at least one of the artificially introduced affinity groups (biotinyl residues}. Hence, the cytotoxic moiety can be removed from the blood circulation even if the immunoconjugate is partly metabolized.

This general concept of catching the immunoconjugates independent of their properties could in principle facilitate the use of a cocktail of different monoclonal antibodies directed against different cell surface antigens. These could later be simultaneously removed from the blood circulation with the one and same adsorption device, provided they are all labeled with the very same affinity group. One major advantage with the use of the combination of biotin/avidin compared to other extracorporeal concepts is its applicability to the processing of whole blood.

A treatment system based on the processing of plasma is quite cumbersome and requires much more advanced equipment than the simpler processing of whole blood. Furthermore, separation of plasma from blood cells through plasma filtration sometimes leads to activation of complement and hemolysis. This is well known in all extracorporeal treatments where plasma separation is used. Hence, all extracorporeal systems based on the processing of plasma require a careful monitoring of infusion of rather large amounts of citrate to the extracorporeal circuit in order to diminish complement activation.

Compatibility with whole-blood processing requires that the immobilized protein does not interact with or activate blood cells and that the binding strength and binding rate constant are sufficiently high. Immobilized antimonoclonal IgG could interact with the F_c receptor of passing immune cells, and the limited binding strength could severely restrict its use in the processing of whole blood, at least when performed at reasonable flow rates. On the contrary, in recent publications (36,40), we have shown that our biotin/avidin concept is also applicable to the processing of whole blood.

Two vascular accesses are mainly needed for an extracorporeal affinity treatment - one for the channeling of blood from the patient to the extracorporeal circuit, and the other for the return of processed blood to the patient. Central vein catheterization is a well-established and widely used procedure to obtain vascular access for extracorporeal treatment. The femoral vein, the subclavian vein, and the external and internal jugular veins have all been utilized for temporary or permanent vascular access. These catheterizations can allow blood flow rates up to 400 mL/min. Citrate and/or heparin are used for anticoagulation of the circuit.

The blood clearance rate is defined as the volume of blood quantitatively depleted of the immunoconjugate per minute, and is expressed as mL/min. In a male patient with a body weight of 75 kg, the blood volume is about 6 L. At a hematocrit of 0.50 the patient has about 3 L of plasma. During a whole-blood adsorption, blood is assumed to be drawn from the patient and passed through the adsorbent at 100 mL/min. At a hematocrit of 0.50, the amount of plasma passing through the adsorbent is 50 mL/min. As the adsorption efficacy is 100%, the blood clearance will be 100 mL/min. In order to process three plasma volumes (9 L), three blood volumes (18 L) has to be processed. The process will require 3 hours of adsorption.

During a plasma adsorption, blood is assumed to be drawn from the patient and passed through the plasma separator at 100 mL/min. As the hematocrit during extracorporeal treatment may not exceed 0.70 in the blood circuit, the maximal tolerable plasma flow rate is 25 mL/min. At this flow rate of blood, the amount of plasma passing through the adsorbent is then 25 mL/min. As the adsorption efficacy is 100%, the blood clearance is 50 mL/min. To process three plasma volumes (9 L), six blood volumes (36 L) has to be processed. The process will require 6 hours of adsorption. Therefore, plasma adsorption will require the double treatment time, and the processed volume of blood will be two times greater.

VII. SUMMARY AND FUTURE

Extracorporeal immunoadsorption, above all whole-blood ECAT, seems to be a both promising and probably clinically feasible tool for improving absorbed dose ratio for tumor to normal tissues. By using biotinylated MAbs and avidin col- umns, there is no need to develop a new adsorbent for each antibody system used in contrast to the use of antiantibody columns. The time for implementation of ECAT is crucial to its success and can be tailored to each antibody employed as to tumor growth kinetics.

Nonmyeloablative (anti-CD20) RIT is well tolerated and appears to be especially effective at inducing durable remissions in a high proportion of patients with low-grade and transformed non-Hodgkin's lymphoma in whom multiple chemotherapy regimens have failed (41). In the more radioresistant de novo inter- mediate and high-grade non-Hodgkin's lymphoma nonmyeloablative therapy seems not equally successful.

Bone marrow reconstitution, transplantation, or peripheral blood stem cell harvest has made it possible to increase the activity of radiolabeled antibody administered. Signs of radiotoxicity in other organs sensitive to radiation (lungs, kidney, and liver) might then appear. Press et al. (42) have used this approach in treating non-Hodgkin's lymphoma with success, but noted lung toxicity when the lung absorbed dose exceeded 25 Gy. DeNardo et al. have demonstrated that patients tolerated higher activities of ^{131}I Lym-1 with less evidence for myelosupression after immunoadsorption than did patients not having immunoadsorption (43).

As ECAT offers the opportunity of administering higher radioactivity by circumventing toxicity in organs sensitive to radiation, future clinical applications of this procedure might be treatment of the less radiosensitive de novo intermediate and high-grade non-Hodgkin's lymphoma.

Only a small percentage of patients with disseminated solid tumors can be cured with current cytostatic regimens. Because solid tumors are far less sensitive to radiation and show more heterogeneous growth than lymphoma, RIT might only be considered when tumor burden is small. Riethmiiller et al. (44) have proven in a randomized study that adjuvant treatment with nonradiolabled MAb 17-1A to patients with colorectal cancer of stage C Dukes' significantly reduced the overall death rate and especially the recurrence rate of distant metastases. The study has now been continued on colorectal carcinoma with proven minimal residual disease in bone marrow.

In light of preceding discussions, RIT might only be considered when the distant cancer metastases show a limited tumor burden, intrinsic radiosensitivity, and feasible antigenic characteristics. In patients with metastases disclosing a fast cell proliferation, repeated RIT and subsequent ECAT with short time intervals (in conjunction with a MAb with fast tumor uptake) should be considered to maintain a high absorbed dose rate in the tumor for a longer time.

The effect of ECA T might be further improved in conjunction with other immunological approaches, e.g., together with cytokines. By combining ECAT with preload (i.e., idiotypic MAb prior to the radiolabeled one), a synergistic improvement of T/N ratios for MAb L6 has been achieved in our experimental model (18).

Buchsbaum et al. (45) have demonstrated that by fractionated RIT a higher concentration of radiolabled MAb was maintained in the tumor periphery for a longer period of time than would have occurred with a single administration.

Thus, both absorbed dose and the dose rate were increased in the proliferating of the tumor. It might be possible that a prior treatment of radiolabled Mab results, for a later treatment with the same radiolabeled Mab, with an accumulation of cells in G2-M phases of the cell cycle (46). By using genetically engineered antibody fragments [preferably $(Fab')_2$] and thereby achieving rapid tumor targeting, repeated ECAT and Mab injections might be employed to further increase tumor activity as well as tumor dose rates. Even when low immunogenic antibodies in a repeated ECAT strategy are used, there is always a risk of evoking antiantibodies in the host. ECAT might however also offer an opportunity to remove human antimouse, human antichimeric, or human antihuman antibodies by passing the patient's blood through the adsorbent column previously saturated with biotinylated antibody.

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REFERENCES

1. Schreiber GJ, Kerr DE. Strategies to enhance the localization of anticancer immunoconjugates. Curr Med Chem 1995; 2:616-629.
2. Wahl RL, Piko CR, Beers BA, Geatti 0, Johnson J, Sherman P. Systemic perfusion: a method of enhancing relative tumor uptake of radiolabeled monoclonal antibodies. J Nucl Med 1987; 28:715. RM 845 J78
3. Wahl RL, Piko CR, Beers BA, Geatti 0, Johnson J, Sherman P. Systemic perfusion: a method of enhancing reative tumor uptake of radiolabeled monoclonal antibodies. Nucl Med Biol1988; 15:611-616.
4. Maddock SW, Maddock EN, Quittner SF, Hamstra A, Pastusiak H. Immunoadsorption of circulating monoclonal antibody. In: 7th International Congress of Immunology. New York: Gustav Fisher Verlag, 1989:771.
5. Strand S, Norrgren K, Ingvar C, Erlandsson K, Persson EC. Plasmapheresis as a tool for enhancing contrast in radioimmunoimaging and modifying absorbed doses in radioimmunotherapy. Med Phys 1989; 16:465. Abstract.
6. Bygren P, Freiburghaus C, Lindholm T, Simonsen 0, Thysell H, Wieslander J. Goodpasture's syndrome treated with staphylococcal protein-A immunoadsorption. Lancet 1985:1295-1296.
7. Palmer A, Taube DH, Welsh K, Bewick M, Gjorstrup P, Thick M. Removal of anti- HLA antibodies by extracorporeal immunoadsorption to enable renal transplantation. Lancet 1989:10-12.
8. du Moulin A. LDL immunoapheresis technique. In: Gotto AM, Mancini M, Richter WO, Schwandt P, eds. Treatment of Severe Hypercholeserolemia in the Prevention of Coronary Heart Disease. Basel: Karger, 1990:170-174.
9. Lear JL, Kasliwal RK, Feyerabend AJ, Pratt JP, Bunn PA, Dienhart DG, Gonzales R, Johnson TK, Bloedow DC, Maddock SW, Glenn SD. Improved tumor imaging with radiolabeled monoclonal antibodies by plasma clearance of unbound antibody with anti-antibody column. Radiology 1991; 179:509-512.
10. Dillman RO. Human antimouse and antiglobulin responses to monoclonal antibodies. Antibody Immunoconj Radiopharm 1990; 3:1-9.
11. Riechmann L, Clark M, Waldmann H. Reshaping human antibody for therapy. Na- lure 1988; 332:323-327.
12. Norrgren K, Strand S, Nilsson R, Lindgren L, Sjogren H. A general extracorporeal immunoadsorption method to increase tumor-to-normal tissue ratio in radioimmu- noirnaging and radioimmunotherapy. J Nucl Med 1993; 34:448-454.
13. Norrgren K, Strand S, Nilsson R, Lindgren L, Lilliehorn P. Evaluation of extracorporeal immunoadsorption for reduction of the blood background in diagnostic and ther- apeutic applications of radiolabeled monoclonal antibodies. Antibody Immunoconj Radiopharm 1991; 4:907-914.
14. Hreitz HB, Weiden PL, Vanderheyden J, Appelbaum JW, Hjorn MJ, Fer MF, Wolf SH, Ratliff HA, Seiler CA, Foisie DC, Fisher DR, Schroff RW, Fritzberg AR, Ab- rams PO. Clinical experience with rhenium-186-1abeled monoclonal antibodies for radioimmunotherapy: results of phase I trials. J Nucl Med 1992; 33: 1099-1112.
15. Camera L, Kinuya S, Garmestani K, Hrechbiel MW, Wu C, Pai LH, McCurry TJ, Gansow OA, Pastan I, Paik CH, Carrasquillo JA. Comparative biodistribution of indium and yttrium-labeled 83 monoclonal antibody conjugated to either IH4M- DTPA or 2H-DOTA. Eur J Nucl Med 1994; 21:640-646.
16. Schuster JM, Garg PK, Higner DD, Zalutsky MR. Improved therapeutic efficacy of a monoclonal antibody radioiodinated using N-succinimidyl 3-(tri-n-butylstannyl) benzoate. Cancer Res 1991; 51:4164-4169.
17. Srivastava SC. Criteria for the selection of radionuclides for targeting nuclear antigens for cancer radioimmunotherapy. Cancer Hiother Radiopharm 1996; 11 :43-50.
18. Garkavij M, Tennvall J, Strand S, Norrgren K, Lindgren L, Nilsson R, Sjogren H. Enhanced radioimmunotargeting of 125I-L6-biotin monoclonal antibody (MAb) by combining preload of cold L6 MAb and subsequent immunoadsorption. Cancer Res 1995; 12:5874-5880.
19. Paganelli G, Stella M, Zito F, Magnani P, De Nardi P, Mangili F, Haratti D, Veglia F, Di Carlo V, Siccardi AG, Fazio F. Radioimmunoguided surgery using iodine-125-1abeled biotinylated monoclonal antibodies and cold avidin. J Nucl Med 1994; 35:1970-1975.
20. Chen JQ, Strand S, Sjogren HO. Optimization of radioiodination and biotinylation of monoclonal antibody chimeric HR96: an indirect labeling using N-succinimidyl- 3-(tri-n-butylstannyl)benzoate conjugate. Cancer Hiother Radiopharm. 1996; 11 : 217-226.
21. Chen JQ, Strand S, Isaksson M, Ljunggren K, Sjogreen K, Garkavij M, Tennvall J, Sjogren H. Hiodistribution and pharmacokinetics of 125-I/131-I pair-labeled, bio-tinylated chimeric HR96 in colon carcinoma isografted rats. Tumor Targeting 1996; 2:204-214.
22. Chen JQ, Strand S, Hrechbiel MW , Gansow OA, Sjogren HO. Combination of bio-tinylation and indium-111labeling with chelate SCN-Hz-CHX-A-DTPA for chimeric HR96: biodistribution and pharmacokinetic studies in colon carcinoma isografted rats. Tumor Targeting 1996; 2:66-75.
23. Chen JQ, Strand S, Tennvall J, Hindorf C, Sjogren H. Biodistribution and phannacokinetics of biotinylated 188Re-chiBR96 in colon carcinoma isografted rats. Tumor Targeting 1998; 3:87-95.
24. Mock DM, Malik MI. Distribution of biotin in human plasma: most of the biotin is not bound to protein. Am J Clin Nutr 1992; 56:427-432.
25. Heard GS, Grier RE, Weiner DL, Secor McVoy JR, Wolf B. Biotinidase-possible mechanism for the recycling of biotin. Ann NY Acad Sci 1985; 447:400.
26. Wolf SB, Hymes I, Heard GS. Biotinidase. Methods Enzymol 1990; 184:103- 111.
27. DeNardo GL, Maddock SW, Sgouros G, Scheibe PO, DeNardo SJ. Immunoadsorption: an enhancement strategy forradioimmunotherapy. I Nucl Med 1993; 34:1020- 1027.
28. Loevinger R, Budinger TF, Watson EE.In: MIRD Primer for Absorbed Dose Calculations. New York: Society of Nuclear Medicine, MIRD, 1991.
29. Johnson TK, Maddock SW, Kasliwal R, Bloedow DC, Hartmann C, Feyerabend A, Dienhart DG, Thickman D, Glenn S, Gonzales R, Lear I, Bunn PA. Radioimmu- noadsorption of KC-4G3 antibody in peripheral blood: implications for radioimmunotherapy. Antibody Immunoconj Radiophann 1991; 4:885-893.
30. Dienhart DG, Kasliwal R, Lear JL, Johnson TK, Bloedow DC, Hartmann C, Selig- man PA, Miller GI, Glenn SD, McAteer MI, Thickman D, Feyerabend A, Maddock EN, Maddock SW, Bunn PA. Extracorporeal immunoadsorption of radiolabeled monoclonal antibody: a method for reduction of background radioactivity and its potential role during the radioimmunotherapy of cancer. Antibody Immunoconj Radiophann 1994; 7:225-252.
31. Schindhelm K. Transport and kinetics in synthetic and immunospecific adsorption columns. Artif Organs 1989; 13:21-27.
32. Sgouros G. Plasmapheresis in radioimmunotherapy of micrometastases: a mathematical modeling and dosimetrical analysis. I Nucl Med 1992; 33:2167-2179.
33. Norrgren K, Strand S, Ingvar C. Contrast enhancement in RI1 and modification of the therapeutic ratio in RIT: a theoretical evaluation of

simulated extracorporeal immunoadsorption. Antibody Immunoconj Radiophann 1992; 5:61-73.

34. Hartmann C, Bloedow DC, Dienhart DG, Kasliwal R, Iohnson TK, Gonzales R, Bunn PA. A phannacokinetic model describing the removal of circulating radiolabeled antibody by extracorporeal immunoadsorption. I Phann Biophann 1997; 19: 385-403.

35. Garkavij M, Tennvall J, Norrgren K, Nilsson R, Strand S, Lindgren L, Sjogren H. Improving radioimmunotargeting of tumors: variation in the amounts of MAb L6 combined with an immunoadsorption system. Acta Onco11993; 32:853-859.

36. Garkavij M, Tennvall I, Strand S, Sjogren H, Chen IQ, Nilsson R, Isaksson M. Extracorporeal whole blood immunoadsorption enhances radioimmunotargeting of 125-I-labeled BR96-biotin monoclonal antibody in syngeneic rat tumor model. I Nucl Med 1997; 38:895-901.

37. Chen IQ, Strand S, Tennvall I, Lindgren L, Hindorf C, Sjogren HO. Extracorporeal immunoadsorption vs. avidin "chase" to enhance tumor-to-normal tissue ratio for biotinylated 188Re-chiBR96. J Nucl Med 1997; 38:1934-1939.

38. DeNardo GL, DeNardo SI, Maddock SW, Zeiter PC, Maddock EN, Matthews KJ. Efficacy of immunophoresis to reduce myelosuppression in radioimmunotherapy. J Nucl Med 1992; 33:863-864.

39. DeNardo SJ, O'Grady LF, Warhoe KA, Kroger LA, Hellstrom I, Hellstrom KE, Maddock SW, DeNardo GL. Radioimmunotherapy in patients with metastatic breast cancer. J Nucl Med 1992; 33:862-863.

40. Garkavij M, Tennvall J, Strand S, Nilsson R, Chen JQ, Lindgren L, Isaksson M, Eriksson H, Sjogren H. Extracorporeal immunoadsorption (ECIA) from whole-blood based on avidin-biotin concept: evaluation of a new method. Acta Onco11996; 53: 309-312.

41. Kaminski MS, Zasadny KR, Francis IR, Fenner MC, Ross CW, Milik AW, Estes J, TuckM, Regan D, Fisher S, Glenn SD, Wahl RL. Iodine-131-anti-BI radioimmunotherapy for B-celllymphoma. J Clin Oncal1996; 14:1974-1981.

42. Press OW, Eary JF, Appelbaum FR, Martin PJ, Nelp WB, Glenn S, Fisher DR, Porter B, Matthews DC, Gooley T, Bemstein ID. Phase n trial of 131-I-BI (anti- CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. Lancet 1995; 346:336-340.

43. DeNardo GL, DeNardo SJ. Treatment of B-lymphocyte malignancies with 131-1- Lym-1 and 67CU-2IT-BAT-Lym-1 and opportunities for improvement. In: Goldenberg DM, ed. Cancer Therapy with Radiolabeled Antibodies. Boca Raton, FL: CRC Press, 1995:217-227.

44. Rietmuller G, Schneider-Gediicke E, Schlimok G, Schmiegel W, Raab R, Hoffken K, Gruber R, Pichlmaier H, Hirche H, Pichlmayr R, Buggisch P, Witte J. Randorni- sed trial of monoclonal antibody for adjuvant therapy of resected Dukes' C colorectal carcinoma. Lancet 1994; 343:1177-1183.

45. Buchsbaum DJ, Khazaeli MB, Liu T, Bright S, Richardson K, Jones M, Meredith RF .Fractionated radioimmunotherapy of human colon carcinoma xenografts with 131-I-labeled monoclonal antibody CC49. Cancer Res 1995; 55:5881-5887.

46. Meredith RF, Khazaeli MB, Liu T, Plott G, Wheeler RH, Russel C, Colcher D, Schlom J, Schochat D, LoBuglio AF. Dose fractionation of radiolabeled antiodies in patients with metastatic colon cancer. J Nucl Med 1992; 33:1648-1653.